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A TECHNIQUE FOR TRAPPING FUNGAL SPORES

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ABSTRACT.—Describes a technique for using petroleum jelly-coated microscope slides to determine the spore dispersal patterns of fungi that cause diseases of nursery, plantation and forest trees.

KEY WORDS.—Disease, pest management, infection, trees, phenology, spore traps.

Early detection, identification, and study of a forest pathogen is essential to prevent a potential epidemic and subsequent economic loss. The biology and life history of a disease-causing organism should be understood, if its controls are to be effective. Spore traps have been used to study the life cycles of fungi and also can be used as a forecasting tool to monitor the presence of certain tree pathogens. The information gained from these traps is extremely useful for planning the control of many forest pathogens. We have successfully used Vaseline[1] petroleum jelly-coated microscope slide spore traps to determine the timing of spore release and dissemination for several serious fungal pathogens in the Lake States. Timing of spore release is particularly important for applying chemical sprays. Knowledge of proper spray timing eliminates extra sprays, which are costly and introduce needless pesticides into the environment.

SPORE TRAP OPERATION

Spore traps are placed in and around infected trees at various heights and distances. These traps are then changed at weekly intervals throughout the season. All slides should be changed on the same day for easy comparison. Potted seedlings, used as exposure trees, can also be placed under infected trees and changed at weekly intervals to determine exact timing of infection. After exposure, the trees are moved to an area free of the disease and observed for the occurrence of disease symptoms. Data from the spore traps and exposure trees can be used to determine when spores are released and when host trees are infected. By graphing spore dispersal data it can be determined when peak amounts of spore inoculum are present (fig. 1-3). Control measures can then be applied prior to and during these peaks to ensure complete protection. Most chemical controls are preventative so must be applied before spores are released to be effective.

The following methods are used to prepare vaseline spore traps for the field and to examine them in the laboratory.

METHODS FOR MAKING VASELINE SPORE TRAP SLIDES

1. Obtain slides that have one frosted end.
2. Fill 2 or more 300 ml beakers two-thirds full of water and place an amount of vaseline in the water so that when it melts there will be approximately a 1-inch layer floating on top. SLOWLY heat the beakers on separate hot plates until vaseline melts to a clear, yellow color. This is usu-

[1] *Mention of trade names does not endorse the products by the USDA Forest Service.*

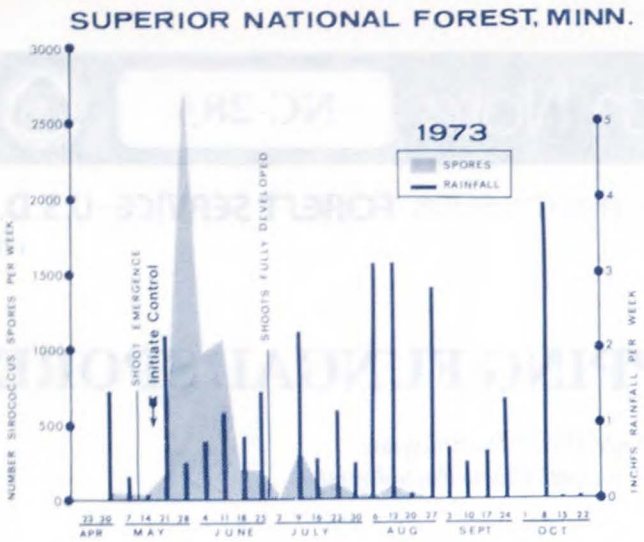


Figure 1.—Sirococcus strobilinus *spore dispersal from red pine correlated with rainfall, Superior National Forest, Minnesota, 1973.*

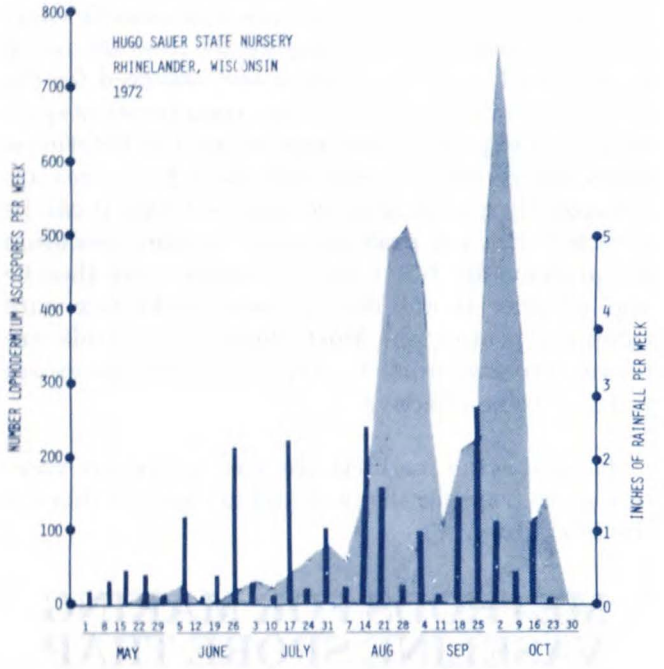


Figure 2.—Lophodermium *needlecast spore dispersal from red pine nursery seedlings correlated with rainfall, Hugo Sauer State Nursery, Rhinelander, Wisconsin, 1972. Note that the major spore dispersal peak for* Sirococcus *shoot blight (fig. 1) is in the early part of the growing season and that for Lophodermium occurs in the latter part of the growing season. Therefore, timing of control measures are different for the two diseases.*

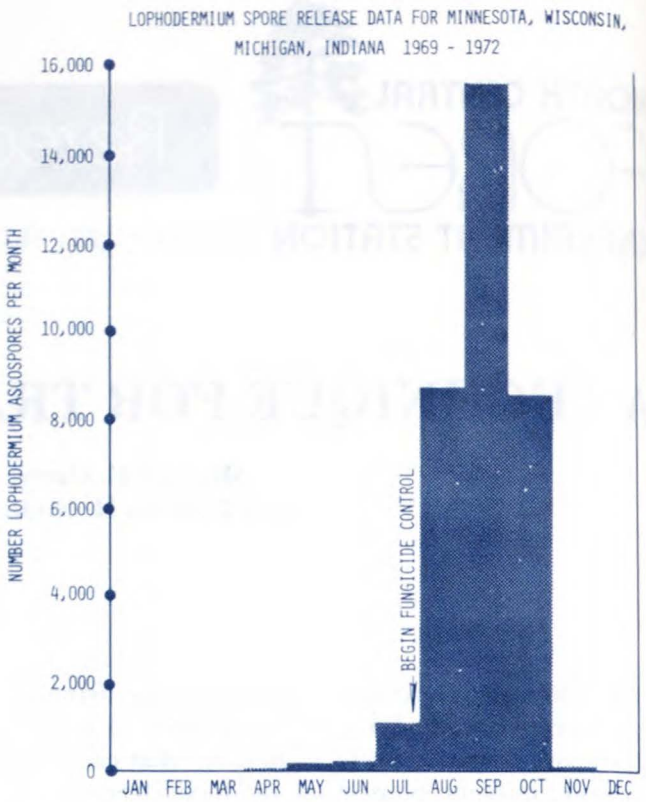


Figure 3.—Lophodermium *spore release data for Minnesota, Wisconsin, Michigan, and Indiana showing how data can be combined to produce a regional spore dispersal graph that can help forest manager's initiate controls at the proper times.*

ally between 65 and 80° C. DO NOT BOIL. Temperature is critical—if it is too cool, vaseline will not adhere smoothly to slides; if it is too hot, vaseline will melt off the sides of slides as they are being dipped. Use several beakers because the water temperature will drop as slides are dipped. Also, more vaseline must be added and melted when the level in the beaker is less than inch thick.

3. Dip two slides at a time. Apply a drop of water to the back of one slide and place it back to back (FROSTED SIDES *OUT)* with another. Holding onto the frosted end, dip the slides into the beaker of melted vaseline using a SMOOTH, vertical motion up to about 1/8-inch from the frosted end. Do not let frosted ends become covered with vaseline. Separate slides and place on a paper towel to cool. Use a spatula to scrape excess vaseline off edges of slides and remove approximately 1/8-inch of vaseline from the end so that it will not smear when the slide is placed in a slide box. The finished slide should have a smooth surface. If it does not, refer to the following trouble shooting guide to determine the cause of the problem.

TROUBLE SHOOTING GUIDE

Problem	Cause	Solution
Vaseline surface lumpy, irregular	Water/Vaseline too cool, jerky motion in dipping slides	Reheat water; dip slides using a smooth, nonstop motion
Vaseline surface too thin or melted off near the edges of slide	Water/Vaseline too hot, dipping too slow so that water heats the slide & melts Vaseline as slide is dipped	Let water/Vaseline cool, dip slides faster
Bubbles on Vaseline surface of slide	Water/Vaseline boiling	Use lower heat setting, let water/ Vaseline cool before using

CAUTION: Do not leave heating beakers unattended; do not boil; use care in handling hot, slippery beakers; do not allow Vaseline to spill onto heating coils. When reheating a beaker of water with Vaseline that has solidified, break up the surface to prevent a boil-over.

4. Label the Slides
   The following is an example of a method that can be used to code exposure slides. Use a hard (4H) lead pencil to avoid smearing.

	Frosted End	—Vaseline—
*	SS 1 4-3-78 4-10-78 A	

KEY
* Solon Springs, Wisconsin, Week 1
Exposed from 4-3-78 to 4-10-78
Spore Trap Location A

5. Keep coated slides in a cool place. They can be stored at room temperature or, preferably, in a refrigerator.
   Exposed slides placed in slide boxes can be sent from field locations to laboratories using carefully padded mailing boxes to avoid breakage.
6. Make Spore trap holders.
   They can be made from aluminum clothesline or similar wire. Bend one length of wire to shape and hold the frosted end of the slide in place by two bends in the wire and by a spring clothespin (fig. 4). For best results in the field, keep slides horizontal and keep vegetation away from the slides. Place slides in and around the areas being monitored. Slides should be positioned under infected trees to ensure trapping spores, especially rain-splash disseminated spores when they are produced. To determine the source or spread of inoculum place slides at known distances in the cardinal directions from infected trees.

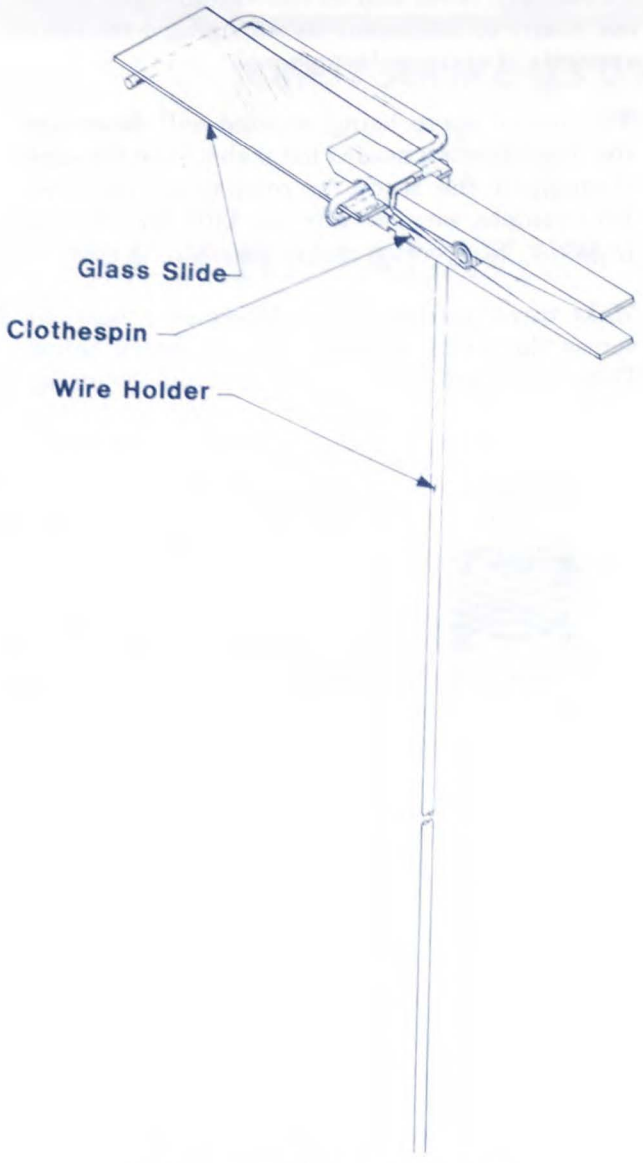


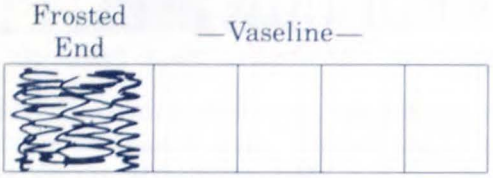
Figure 4.—*Spore trap holder.*

7. Read the slide with a microscope.
   A cover slip is not necessary if a stain is not used except at high magnifications where it is helpful in preventing vaseline from accidently smearing the objective. The use of a stain is necessary when counting hyaline or other light-colored spores. Staining can usually be done by placing one drop of lactophenol and one drop of stain in the middle of each transect to be counted. Then gently place

a 24x50mm cover slip on the vaseline and allow the stain to disperse. Avoid using extensive amounts of stain or lactophenol.

The size of spore being counted will determine the magnification needed but it should be the same throughout the study for comparison purposes. For example, we generally use 430X for counting *Diplodia*, *Sirococcus*, and *Gremmeniella* spores.

Make three transects at predetermined locations across the width of each slide as shown below. Then count and total all spores in each transect.



Spore Count Transects

MONITORING WEATHER AND PHENOLOGY

The development and severity of disease is greatly affected by weather conditions. To study this, we maintain weather stations at each study site where spore traps are located. Each station consists of a weather shelter containing a temperature-relative humidity 7-day recording hygrothermograph and a rain gauge. The phenological development of parasite and host are monitored at weekly intervals using a standard field form. Shoot and foliage elongation and presence of fruiting bodies or needlespots are recorded and correlated with weather and spore release data.

CONCLUSION

The techniques described in this paper can be used to inexpensively provide knowledge on the biology of a fungus pathogen that is needed to help make accurate control judgments. Control recommendations for one part of the country may not necessarily work for the same species in other areas because of climatic or physiological and genetic differences in hosts and pathogens. Therefore, it may be necessary to adjust timing of control practices for different areas. These adjustments can be made easily and quickly by obtaining the necessary information using these techniques.

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